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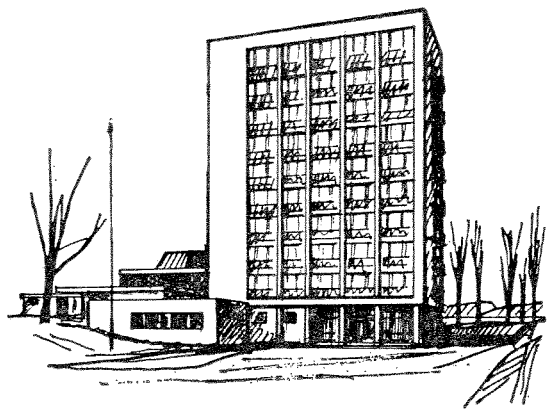

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COMPARISON OF BLOOD PROTEINS FROM EAST AND WEST ATLANTIC POPULATIONS OF *HIPPOGLOSSOIDES PLATESSOIDES*

By

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ABSTRACT

NÆVDAL, G. and BAKKEN, E. 1974. Comparison of blood proteins from east and west Atlantic populations of *Hippoglossoides platessoides*. *FiskDir. Skr. Ser. HavUnders.*, *16*: 183–188.

Hemoglobins, serum proteins and serum esterase of *Hippoglossoides platessoides* from the eastern and western part of the North Atlantic were analyzed by gel-electrophoresis. Great variety were observed among specimens, and a few rare phenotypes were found only among representatives of one of the two areas. Most phenotypes, however, were found both in the east and west Atlantic samples, although they occurred at different frequencies. The observed differences give no basis for regarding the east and west Atlantic populations as separate species.

INTRODUCTION

NORMAN (1934) divided the species *Hippoglossoides platessoides* (Fabricius) in the North Atlantic into two subspecies, each with its own geographical range: *H.p. limandoides* (Bloch) in northwestern Europe and *H.p. platessoides* (Fabricius) in North America. He points out, however, that the European and American forms, called long rough dab and American plaice respectively, intergrade in areas where their ranges overlap. Specimens from Iceland and Spitzbergen, for example, approach the American subspecies in depth of body, number of scales, etc.

H. platessoides is only lightly exploited but may be regarded as a potential fish resource both on the east and west side of the North Atlantic. For management purposes criteria for distinction between possible stock units will be of significance.

The purpose of the investigations reported here has been to study the relation between the two subspecies by use of characteristics of some blood proteins.

MATERIAL AND METHODS

An account of the collected material is given in Table 1.

Samples of the fish were selected to cover the entire size range. Bloods were obtained by cutting the tail or drawn by syringe from the heart.

Table 1. Samples of *Hippoglossoides platessoides* analyzed for blood protein variations.

Sample no	Locality and date	Length range, cm	Specimens analyzed		
			Hemoglobin	Serum proteins	Serum esterase
1	St. Margaret's Bay, N.S., Canada Apr '68	28—53			14
2	— Oct '68	29—59	73	73	73
3	59°20'N 04°00'E, North Sea Aug '68	12—20	60	10	51
4	72°00'N 30°00'E, Barents Sea May '70	25—40		90	90

Samples from Canada were shipped by air to the Institute of Marine Research, Bergen and received within two days. Sample 1 was sent as whole bloods, but this caused lysis of the blood cells, and the sample was suitable only for analyses of serum esterase. In sample 2 sera were separate from the cells before shipping, and both sera and cells were received in good conditions.

Sample 3 was collected onboard a trawler and sent to the Institute where it was received the next day. Sample 4 was collected onboard R.V. "Johan Hjort". This sample had to be kept in the deep freeze until the ship returned to Bergen, and because fish hemoglobins withstand freezing poorly, only sera were analyzed.

Hemoglobins were analyzed by agar-gel electrophoresis at pH 7.2 as described by SICK (1965). Sera were analyzed by the combined starch and agar-gel electrophoresis described by MØLLER (1966) and stained for general protein patterns by Nigrosin and for esterase activity by α-naphtylacetate with Fast Blue BB Salt as dye coupler.

Hemoglobins were analyzed fresh while sera were analyzed both fresh and after being kept in the deep freeze for several months.

RESULTS

HEMOGLOBINS

The observed hemoglobin patterns are outlined in Fig. 1 A.

Two strong and one weak band, pattern 1, were found for all specimens, except four which had individual patterns. Patterns 2 and 3 were found in the sample from the North Sea while 4 and 5 were found in sample 2 from Canadian waters.

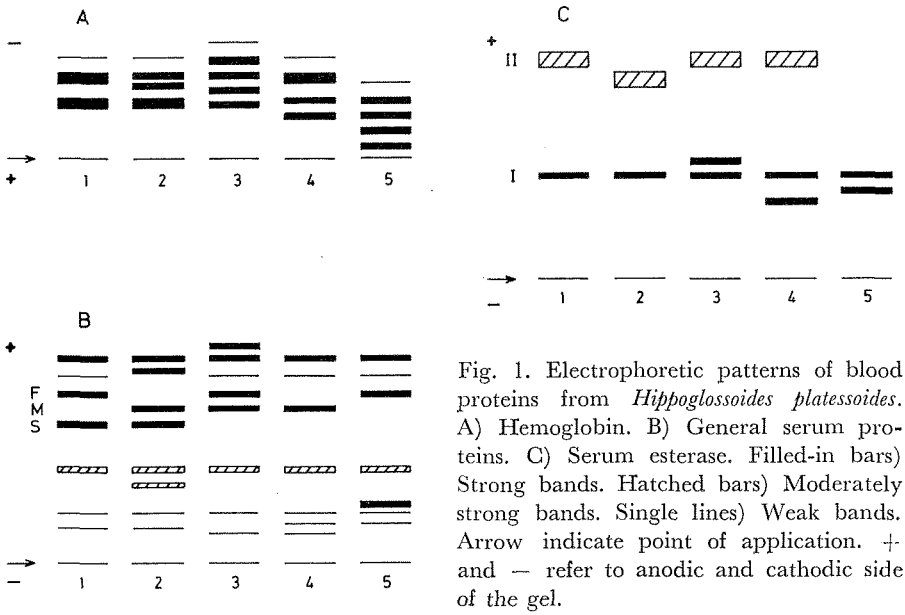

Fig. 1. Electrophoretic patterns of blood proteins from *Hippoglossoides platessoides*. A) Hemoglobin. B) General serum proteins. C) Serum esterase. Filled-in bars) Strong bands. Hatched bars) Moderately strong bands. Single lines) Weak bands. Arrow indicate point of application. + and — refer to anodic and cathodic side of the gel.

The observed variation may be genetically controlled, but this hypothesis can not be tested on the present material due to the scarsity of other phenotypes than the "normal" one.

GENERAL SERUM PROTEINS

Some typical patterns of general serum proteins are outlined in Fig. 1 B. A high degree of variation among individual specimens was found within all samples.

The proteins of highest anodic mobility, the albumins, were seen as a single band in most specimens, sometimes with a weak postalbumin at its cathodic side. Double albumins, patterns 2 and 3, occurred at low frequencies both in samples from the west and east Atlantic.

At least three strong bands, called F(ast), M(iddle) and S(low), occurred at the cathodic side of the albumins. In all specimens one or two of these bands were seen, indicating control by three (or more) allelic genes. However, these bands were not always clear enough to permit calculations of frequency distributions of the phenotypes, and thus the hypothesis of genetic control could not be tested. The S band occurred at considerably lower frequency in the samples from the east compared to the west Atlantic.

Individual variations, probably genetically controlled, were observed in several groups of weak components with low anodic mobility.

Due to the weakness of the bands, grouping of the individuals on the basis of their variations was impossible.

In the sample from the Barents Sea one strong component occurred in some specimens; pattern 5. This band may represent the "ripe female protein" noted in other species (NÆVDAL 1969, TSUYUKI and ROBERTS 1966).

SERUM ESTERASE

The patterns of esterase activity are outlined in Fig. 1 C.

In the Canadian samples two main zones of esterase activity, called I and II, occurred. In two specimens the II band was lacking. In the samples from the east Atlantic the II band was observed in only four specimens. When the II band occurred, it was found at the same position as in the Canadian samples, except in one specimen where it was found to possess somewhat lower anodic mobility; pattern 2.

Three different variations of double I bands were found; patterns 3, 4 and 5. Three specimens of sample 2 and one specimen in each of samples 3 and 4 showed an extra band at the anodic side of the normal I band. Extra bands at the cathodic side of the normal I band were seen at two positions. The slower moving band, pattern 4, was found in one specimen of sample 1 and five specimens in each of samples 3 and 4. An extra band of somewhat higher mobility, pattern 5, was only found in two specimens of sample 4.

A genetic system of four alleles would explain the observed variation of the I band, but scarsity of the variants, hypothetical heterozygotes, prevents this hypothesis from being tested by population data.

DISCUSSION

Two populations are said to be conspecific when they are actually or potentially inbreeding (MAYR, LINDSLEY and USINGER 1953). The populations of *H. platessoides* from the east and west Atlantic are geographically isolated and consequently not actually inbreeding. The problem of their conspecific nature therefore is reduced to determine whether they are potentially inbreeding.

In the present study the genetic basis of the observed variation has not been worked out in details. Bur no indications of growth dependent variation were found, and sex dependent variation was only indicated in one serum protein of low anodic mobility. The possibility exists that modifications caused by factors other than genetic may account for part of the observed variations. However, the genetic basis of the protein structure (PEACOCKE and DRYSDALE 1965) strongly signify that analyzes of charac-

teristics of the proteins are useful for discrimination of the genotype of individuals and populations.

The east and west Atlantic populations did not differ to a great extent. In the characteristics studied here some hemoglobin and serum esterase I phenotypes were found among representatives of one population only. All these phenotypes were rare, and analyses of greater material would possibly show that they exist also in the other population.

The greatest difference was found in the esterase II component which was lacking in most specimens from the east Atlantic. However, some specimens contained this component as well as it infrequently was lacking in the west Atlantic samples. The varied occurrence of esterase II is thus merely a difference in frequency distribution of phenotypes. Such differences were also observed in distributions of some esterase I components and serum protein components.

The two subspecies, as established by NORMAN (1934), differ in some morphological and physiological characters. In European waters (Clyde area) the maximum length is about 30 cm at 6 years of age (BAGENAL 1955) while it is about 68 cm and 26 years in Canadian waters (POWLES 1965). The fecundity or rate of egg production in relation to size and age is remarkably similar (PITT 1964), but the females of the European form mature at much smaller size and at much younger age than the American form.

Such physiological variance are commonly found between fish subspecies and correspond to the differences observed in the present investigation of blood characteristics. These differences are of a type which should be expected between conspecific populations rather than between species.

The results of the present study have not excluded that the east and west Atlantic populations of *H. platessoides* is potentially inbreeding, and until greater differences in their genotypes are revealed, they should be regarded as conspecific.

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A TECHNIQUE FOR SECTIONING BLUE WHITING OTOLITHS FOR AGE DETERMINATION

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ABSTRACT

JAKUPSSTOVU, S. H. í. 1974. A technique for sectioning blue whiting otoliths for age determination. *FiskDir. Skr. Ser. HavUnders.*, *16*: 189—193.

The basic principle of the method is to embedd the otoliths in a two component glue whereafter it is sectioned with a jewelers saw. When using this method a trained technician can section and make age determinations of approximately 50 otoliths per day.

INTRODUCTION

Age determination on blue whiting, *Micromesistius poutassou* (Risso, 1810), is most successfully performed by counting alternating hyaline and opaque zones in the otoliths (RAITT 1968).

The method commonly used in examining blue whiting otoliths is that discribed by GAMBELL and MESSTORFF (1964) for whiting otoliths. The otolith is broken transversely, and a beam of light striking the side of it is transmitted upwards through it, illuminating the hyaline and opaque zones on the broken surface. When working on blue whiting from the Norwegian Sea and adjacent areas this method was found to be difficult in obtaining reliable age determination of fish older than 5—7 years. The reason for this is that the outer opaque zones in old fish are very thin, and a great proportion of the fish form secondary rings in the otoliths. When sending the light in from the side the illumination of the surface is in many instances insufficient to distinguish primary rings from secondary rings, and the contrast is too weak to permit a sufficient discrimination of the zones.

In order to obtain better contrast in the otoliths, burning (CHRISTENSEN 1964) and dying (ALBRECHTSEN 1968) the otoliths has been tried without success. Sections are the only means by which the internal structure of some otoliths can be seen (JOHNSTON 1938). The main objec tions against sectioning otoliths is the time used for preparation of sections. In the search for an efficient method a modification of that described by

TÅNING (1938) for cutting cod otoliths has been adopted. This is a fairly rapid method and a trained technician can prepare and determine age of about 50 otoliths per day.

THE METHOD

The basic principle of this method is to embedd the otolith in a plastic glue whereafter it can be cut into sections with a saw. The embedding medium used is a two component glue (Araldit, manufactured by A/S Sigurd Hesselberg, Oslo). This gives a firm grip on the otolith and prevents breaking of the sections when sawed.

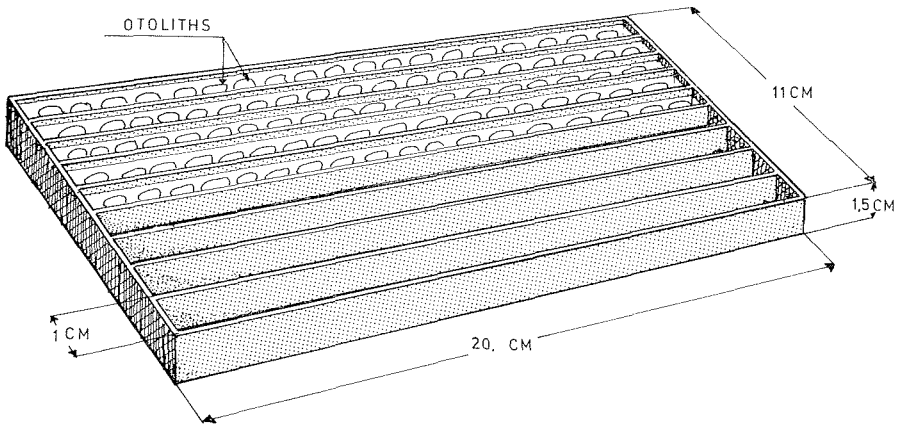

Fig. 1. Sketch of the tray-mould used for embedding the otoliths.

For practical reasons a special tray-mould has been made for the embedding (Fig. 1). This is made of metal and divided into 9 compartments, each giving room for 12—15 embedded otoliths. Before embedding the inside of the mould is covered with a thin layer of paraffin wax in order to prevent the glue from sticking to it. After this a ground layer (1—2 mm thick) of Araldit is placed in the form and allowed to dry for 1—2 hours at room temperature. The glue is then stiff enough to prevent the otolith from sinking, and at the same time it holds the otoliths when they are finally covered with glue.

A 1—3 mm thick cover of glue is sufficient to hold the otoliths during sawing, and when using this thickness the otoliths are fully visible, making it possible to section any desirable place of the otolith.

When the glue is dry (24—48 hours at room temperature, earlier if heated), the mould is heated to above the melting point of the paraffin.

The blocks of Araldit with the embedded otoliths can then easily be taken out, and in this form they can be kept indefinately.

The sectioning is done with a jewelers saw. The best results have been obtained with steel blades 0.2 mm thick and with 20.3 teeth per cm. Good results have also been obtained with thicker blades, but the disadvantage with these are that a greater part of the otolith is lost during sectioning.

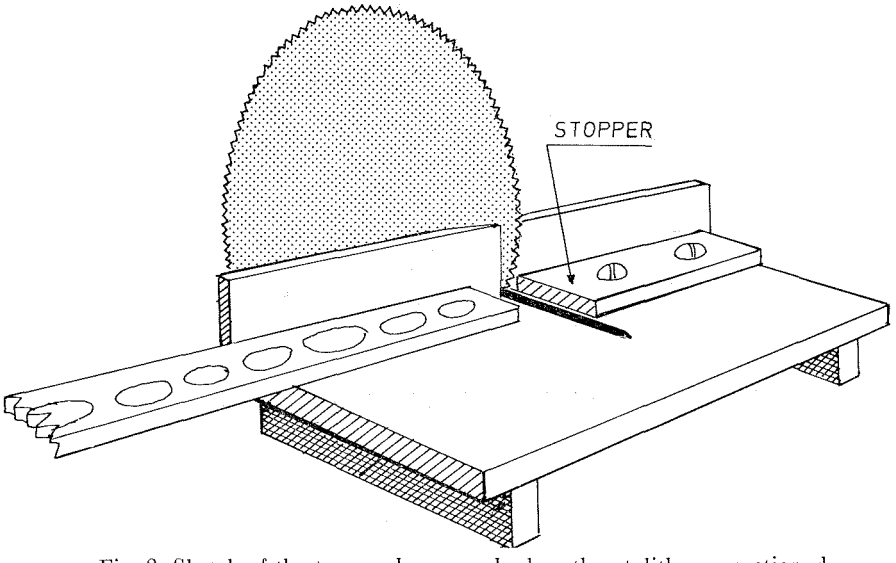

Fig. 2. Sketch of the tray and saw used when the otoliths are sectioned.

The embedded otoliths are placed on a tray with an adjustable stopper (Fig. 2) and held firmly by hand. By moving the tray against the rotating blade sections down to 0.3 mm can be made. The preferred thickness is form 0.4—0.6 mm.

Blue whiting has large otoliths with a wide first zone, and 2—3 sections can usually be made from the centre area without losing any zones. The sections are then washed in alcohol, which also acts as a clearing agent, and mounted in Eucit on glass slides.

The reading can be done immediately by binocular microscope with transmitted light from below, using a grey filter. A better result is obtained with a polarisation filter which makes it possible to change the angles of the light waves against the zone walls.

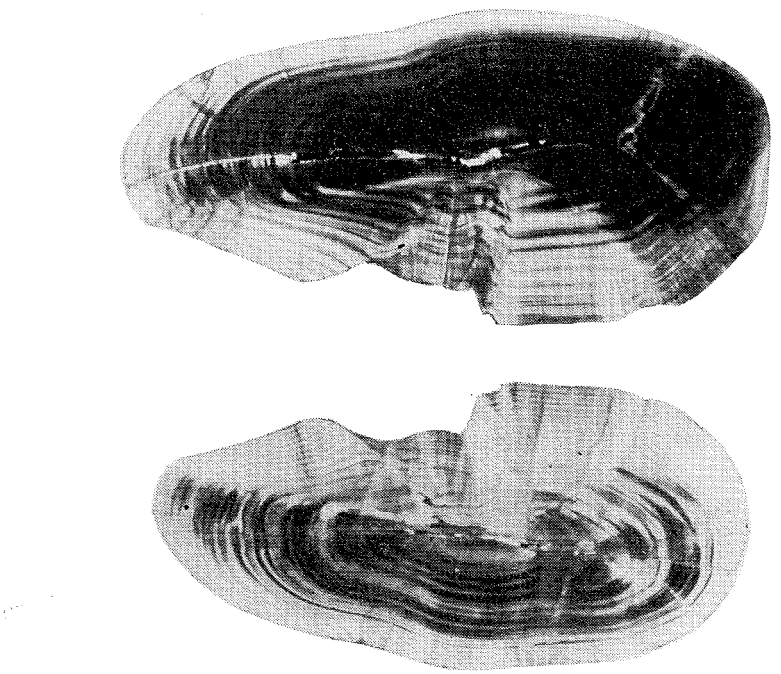

Fig. 3. Sections from blue whiting otoliths. Top) Female 32,5 cm. Bottom) Female 33,5 cm.

RESULTS

So far, the results of the sectioning seem very promising. Sections have been made from several hundred otoliths, and age determinations have been possible for otoliths formerly assigned as unreadable or uncertain. Fig. 3 illustrate sections from two otoliths.

The method has also been tried on otoliths of polar cod with good results.

ACKNOWLEDGEMENT

The method is based on equipment used for sectioning seal teeth, and I am indebted to T. ØRITSLAND and B. BERGFLØDT for their kind help. Special thanks is due to K. A. LARSEN who has prepared and photographed the sections and H. KISMUL who has drawn the illustrations.

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ON THE HYDROGRAPHIC FLUCTUATIONS IN THE LABRADOR SEA DURING THE YEARS 1959—1969

By

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ABSTRACT

BLINDHEIM, J. 1974. On the hydrographic fluctuations in the Labrador Sea during the years 1959—1969. *Fish. Dir. Skr. Ser. Hav Unders.*, *16*: 194—202.

During the years 1959—1969 Norwegian fishery research vessels collected oceanographic data off West Greenland, the observational work mainly being done in April. The data reveal gradually falling temperatures in the upper layers because of increasing supply of Arctic water to the West Greenland Current during the 1960s. The reason for this seems to be the atmospheric pressure and wind conditions which also were in favour of an offshore drift of the surface waters along the West Greenland coast.

Between the surface waters of Arctic characteristics and the Irminger water below vertical convection was to a great extent prevented by a transition layer of high stability. The radiant heat loss during the winter season was, therefore, limited to the upper layers, adding to the decrease in temperature created by the growing supply of Arctic water. In waters of salinity below 34‰ in the section across the Fylla Bank the temperature fell by 1.6°C during the period.

The trend in the Irminger water was different, and the temperature in this water mass rose until 1966.

The cooling in the upper layers can be traced down to approximately 400 m depth, but it was most pronounced at 100 to 150 m depth off the edge of the shelf where the temperature fell by about 2°C. At depths below 400 m the warming effect of the Irminger water can be traced down to 1 000 to 1 200 m depth.

INTRODUCTION

In West Greenland waters where some important fish species find the poleward border of their range, these species are particularly sensitive to environmental fluctuations. This was clearly demonstrated at the beginning of the last good cod period on the West Greenland fishing banks when a marked temperature increase was observed. The longest series of oceanographic observations in the area are sea surface temperatures collected for the Danish Meteorological Institute since 1876. As shown by SMED (1965) these observations indicate a marked tempera-

ture increase in the 1920s. The warming continued until the beginning of the 1930. Since then the temperature has on an average remained above the mean for the 40 year period 1876—1915 until recently.

The biological consequences of this temperature increase is described by JENSEN (1939), the best known and most important being the northward extention and strongly increased abundance of the cod stock. The capelin in the area showed an opposite reaction as the southern limit of its range shifted northwards.

Since the beginning of the 1960s a cooling trend has been observed, and the temperature in the surface layer is now about the same as it was before 1920, and its unfavourable effect on the cod stock is already being observed. The reason for this is a general climatic deterioration over the Northwest Atlantic and the European Arctic. This hydro-meteorological fluctuation has in particular been studied by RODEWALD (1967, 1969, 1971), and he shows that since the 1950s the atmospheric pressure in the Greenland high has been above normal. This has given rise to anomaly northerly winds over the Greenland and Norwegian Seas, and consequently to a temperature decrease in this area. It has also given rise to increased transport of Polar water to the East Greenland Current, and as described by MALMBERG (1969), also to the East Icelandic Current. In the North Atlantic south of Iceland and in the Irminger Sea the anomaly pressure and wind have caused an increased supply of Atlantic water to the Irminger Current. Off West Greenland the atmospheric conditions brought about an offshore drift of the surface waters which resulted in an abnormal great lateral extent of the Arctic Component of the West Greenland Current. As reported on by BLINDHEIM (1967) this is demonstrated by observations made on Norwegian fishery research vessels which surveyed the area during the spring of the years 1959—1969. After 1969 these investigations were discontinued and the present paper is, therefore, based on the complete series of hydrographic data from these cruises.

MATERIAL AND METHODS

The hydrographic data consists of temperature and salinity observations made with Nansen casts. Most of them are collected in late March or in April, and some few in the first days of May. Mainly due to ice obstacles, the station grid varied somewhat from year to year. In general, however, the intention was to work sections perpendicular to the coast across the more important fishing banks. As an example the grid of stations worked in 1966 is shown in Fig. 1. The number of stations worked in the sections across the banks in the different years are compiled in Table 1.

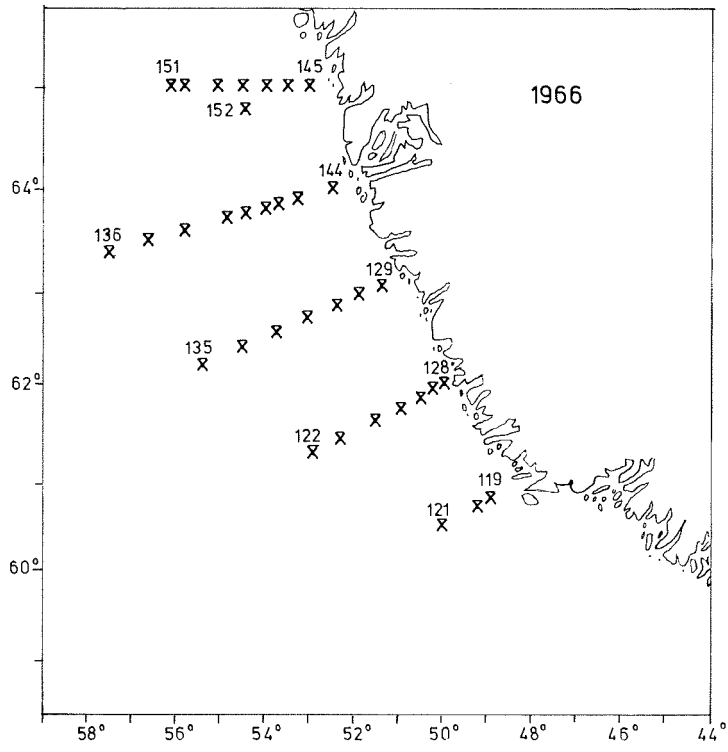

Fig. 1. Grid of stations worked in 1966.

Table 1. Number of hydrographic stations worked in the different sections across the fishing banks off West Greenland in the years 1959—1969.

Year	Fredriks-håb Bank	Danas Bank	Fyllas Bank	Lille Hellefiske Bank	Other	Total
1959 . . .	4	8	8	8	10	38
1960 . . .	5	5	5	5	8	28
1961 . . .	7	7	7	7	25	53
1962 . . .	4	0	5	5	2	16
1963 . . .	6	0	9	5	7	27
1964 . . .	7	6	6	5	15	39
1965 . . .	6	5	9	6	9	35
1966 . . .	7	7	9	7	4	34
1967 . . .	7	7	5	4	6	29
1968 . . .	5	7	9	7	5	33
1969 . . .	0	7	8	6	4	25

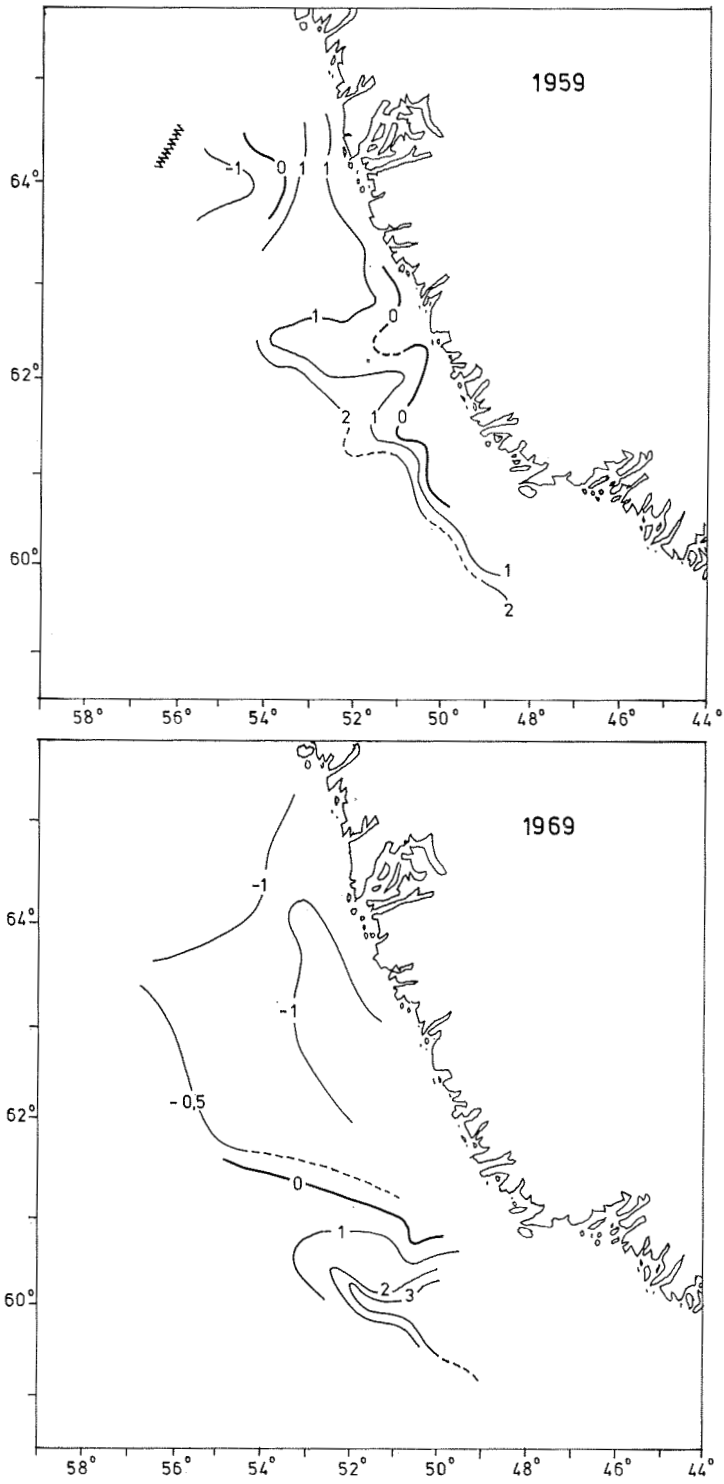

Fig. 2. Surface temperature as observed in April 1959 (top) and April 1969 (bottom).

RESULTS AND DISCUSSIONS

Fig. 2 shows the distribution of the surface temperature as observed in April 1959 and in 1969. Even though the chart for 1969 is lacking somewhat in detail, it is evident that the temperatures in the surface layer have decreased considerably during the period. In 1959 only a minor area close to the coast exhibited temperatures below 0°C while in 1969 the isotherm for 0°C was situated far from the coast, and the greater part of the survey area had sub-zero temperatures in the upper layers. By comparing similar charts for all years during the period it is seen that from 1961 the lateral extent of the Arctic Component of the West Greenland Current has increased gradually.

Sections across the Fylla Bank from 1961 and 1969 are shown in Fig. 3 and Fig. 4 respectively. They demonstrate the situation typical for the beginning and the end of the period. The increased lateral extent of the waters of Arctic and coastal origin in the West Greenland Current towards the end of the period is clearly seen. In the section from 1961 only the waters close to the coast was colder than +1°C with associated salinities about 34.1‰. In 1969 the temperature was below 0°C in the upper 52 m along the whole section, and the associated salinities had

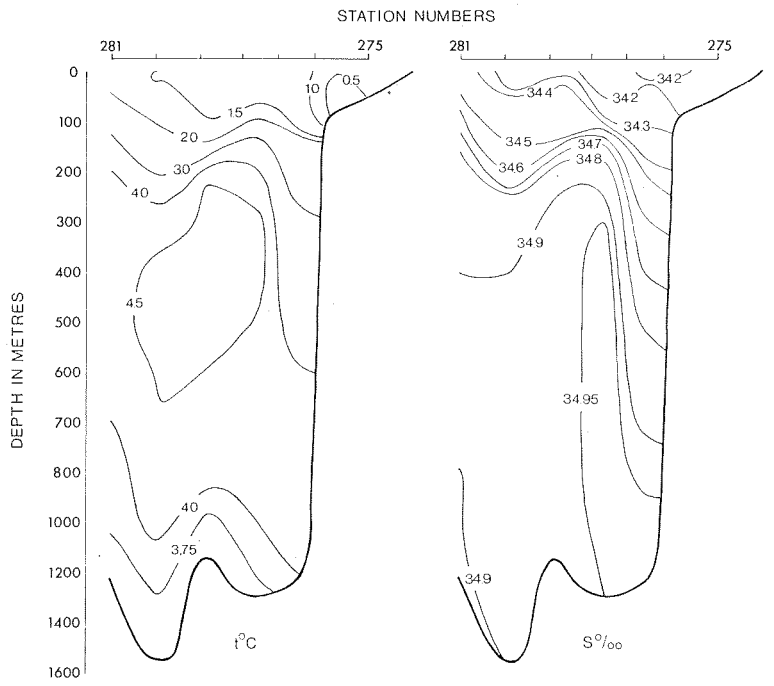

Fig. 3. Temperatures and salinities in a section across the Fylla Bank in April 1961.

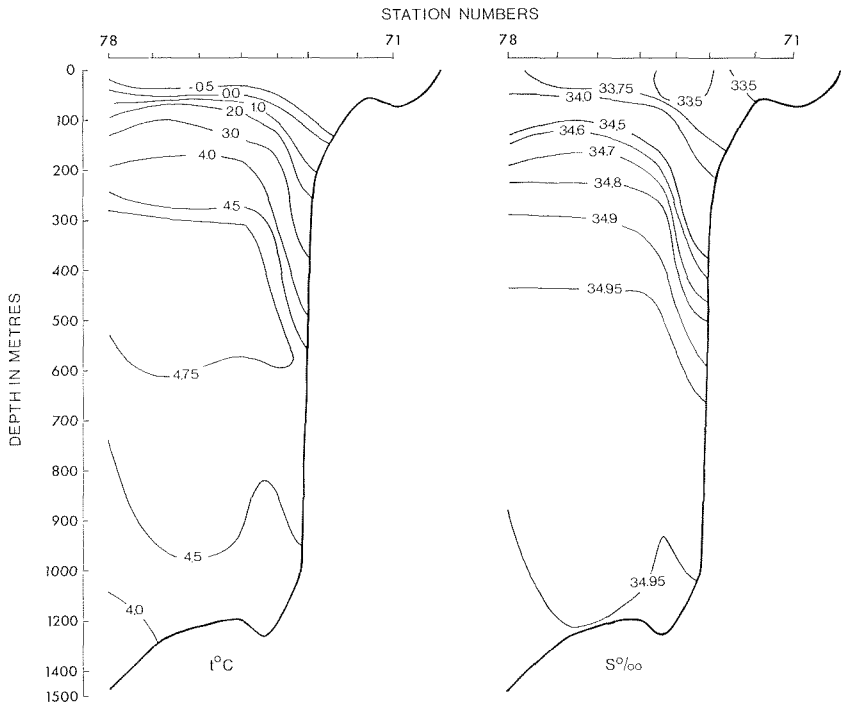

Fig. 4. Temperatures and salinities in a section across the Fylla Bank in April 1960.

decreased to between 33.5 and 33.8‰. The temperature decrease in the upper layers during the 11 year period is demonstrated by the curves in Fig. 5. The figure shows three years moving average of mean temperature and salinity in the Arctic Component of the West Greenland Current, i.e. in waters of salinity below 34.0‰. The figure shows that the temperatures have fallen gradually during the period and the difference between the mean for 1959 — 61 and 1967 — 69 amounts to 1.6°C. In the same manner the curve for the salinity shows a decrease of about 0.3‰, but during the last years of the period it shows a minor increase.

The Irminger Component of the West Greenland Current is seen in Fig. 3 and 4 as a temperature maximum at depths between about 300 and 600 m. It is shown in the figures that this water mass exhibited somewhat lower temperatures in 1961 than in 1969, the maximum being about 4.6 in 1961 and between 4.9 and 5.0°C in the end of the period. Fig. 6 which shows mean temperatures between 300 and 500 m depth in the Fylla sections, demonstrates the temperature trend in the Irminger Component more in detail. The mean feature demonstrated is a relatively cold period in the years 1961, 62 and 63 followed by an increase which culminated in 1966 when the mean temperature was 1.4°C

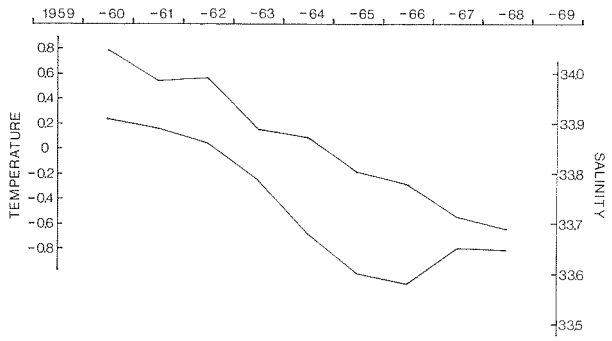

Fig. 5. Three years moving averages of temperature and salinity in waters of salinity below 34‰ in the section across the Fylla Bank.

above the mean value for 1963. During the following years the temperature decreased again to values below the overall mean for the 11 year period. A similar trend has also been observed in the other sections to the north and south of the Fylla Bank. The temperature increase until 1966 is in agreement with the trend in the July temperature which is reported on by HERMANN (1967).

A trend which is closely related to the trend in the Irminger water off West Greenland is also observed in the surface layer at OWS ALFA in the Irminger Sea as described by RODEWALD (1971). The fluctuations observed in the Irminger water of the West Greenland Current are, therefore, related to the conditions in the Irminger Sea. The reason is, as explained by RODEWALD (1971), the distribution of the atmospheric pressure and wind field which up to the mid 1960s were in favour of greate transport than normal of Atlantic water to the Irminger Current. At the same time, however, the supply of Arctic water to the East-, and consequently to the West Greenland Current was also enlarged and contributed to its increased offshore extent in the surface layer.

The decreasing salinity in the surface layer brought about a decline

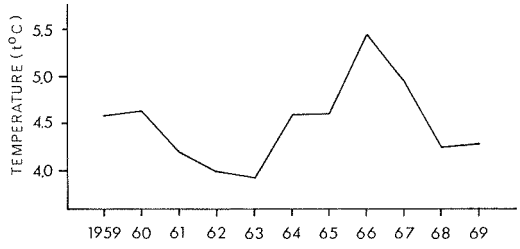

Fig. 6. Mean temperature between 300 and 500 m depth in the section across the Fylla Bank.

in density, the lowest values being observed in 1966, but densities remained low also during the rest of the period. In 1966 the mean δ_t-value in the upper 50 m of the Fylla section was 0.5 less than the value for 1960. Consequently the transition layer between the more or less Arctic waters of the surface layer and the Irminger water below became more pronounced, and the vertical exchange of heat by convection was consideraly reduced. During the winter season this resulted in an additional cooling of the surface layer because of radiant heat loss and only moderate heat supply from below. This seems also to be the reason for rising maximum temperatures in the Irminger Component from south to north. In the section across the Lille Hellefiske Bank the average maximum temperature in the Irminger water during the period was 5.11°C. This is respectively 0.22°C and 0.48°C higher than the associated averages in the sections across the Fylla and Dana Banks further south. A similar, but not so pronounced trend was also established for the salinity. This northward augmented preservation of the Irminger characteristics may be explained by a northward decrease in vertical convection during the winter season.

The vertical distribution of the temperature and salinity fluctuation is illustrated in Fig. 7. This figure shows a mean difference between the

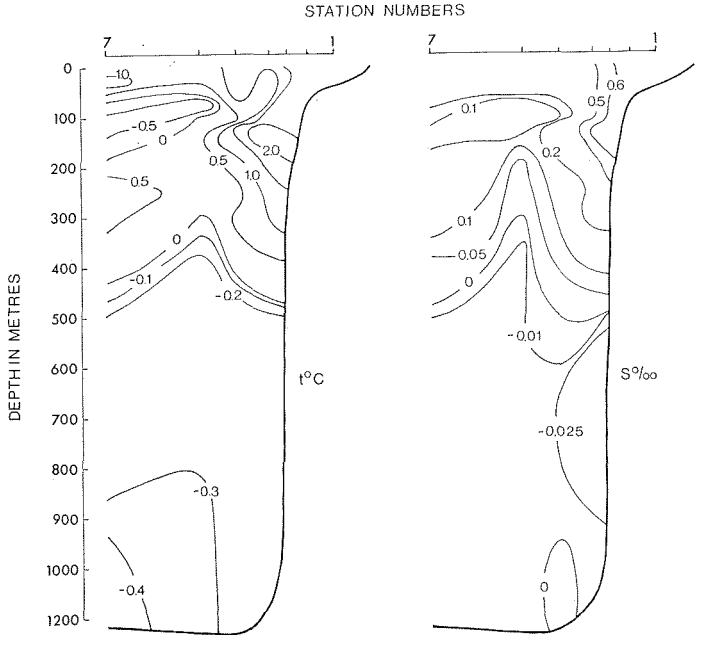

Fig. 7. Vertical distribution of differences in temperature and salinity between means for 1968—69 and 1959—61 in the section across the Fylla Bank.

beginning and the end of the period in the section across the Fylla Bank. It is based on the sections worked in 1959, 1961, 1968 and 1969. The temperature differences, t, which are depicted in the section are

$$t = \tfrac{1}{2}(t_{1968} + {}_{1969}) - \tfrac{1}{2}(t_{1959} + t_{1961}),$$

and the salinity values are worked out in the same manner. The figure indicates that the temperature has increased down to about 400 m depth, the cooling effect being most pronounced over the shelf and slope. At 150 m depth just off the edge of the shelf the difference exceeded 2.0°C, the associated difference in salinity being between 0.6 and 0.7‰. The extent of Arctic water which spread out in the upper layers during the period, is indicated by a difference of about 0.5°C and 0.2‰ above approximately 50 m depth.

In 1968 and 1969 the transport of Irminger water to the Labrador Sea was diminishing compared to the inflow in the middle of the period as indicated in Fig. 6, but still it's temperature was higher in the beginning of the period. This is shown in Fig. 7 by the core which is indicated by rising temperatures at intermediate depths. It is also seen that the effect of the Irminger water, being mixed with surrounding water masses, can be traced down to depths of 1 000 to 1 200 m.

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